EGFR signaling in renal fibrosis

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Signaling through the epidermal growth factor receptor (EGFR) is involved in regulation of multiple biological processes, including proliferation, metabolism, differentiation, and survival. Owing to its aberrant expression in a variety of malignant tumors, EGFR has been recognized as a target in anticancer therapy. Increasingly, evidence from animal studies indicates that EGFR signaling is also implicated in the development and progression of renal fibrosis. The therapeutic value of EGFR inhibition has not yet been evaluated in human kidney disease. In this article, we summarize recent research into the role of EGFR signaling in renal fibrogenesis, discuss the mechanism by which EGFR regulates this process, and consider the potential of EGFR as an antifibrotic target.

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The epidermal growth factor receptor (EGFR/HER1) belongs to a receptor tyrosine kinases protein family that includes HER2/*neu*, HER3, and HER4.[1] At least six EGFR ligands, including transforming growth factor-α (TGF-α), epidermal growth factor (EGF), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, betacellulin, and epiregualtin, have been identified in kidney cells.[2–5] Depending on the activating ligand, EGFR family members form homodimers or heterodimers with different biological effects.[6] Activation of EGFR can lead to diverse cellular consequences associated with fibrogenesis, including cell proliferation, migration, differentiation, survival, and transformation.[7–8]

EGFR can be activated by several mechanisms under physiological or pathophysiological conditions. Like other tyrosine kinase receptors, EGFR is directly activated by ligand binding, inducing activation of the intrinsic kinase domain and subsequent phosphorylation of specific tyrosine residues. These phosphorylated residues initiate activation of intracellular pathways, including the extracellular signal-regulated kinases1/2 (ERK1/2), the Janus kinase/signal transducers and activators of transcription (STAT), and the phosphatidylinositol-3 kinase/AKT.[1,7,9] On the other hand, EGFR can be transctivated by stimuli that do not directly interact with the EGFR ectodomain, including G protein–coupled receptor ligands (i.e., endothelin, lysophosphatidic acid, thrombin),[10] cytokines (i.e., interleukin-8),[11] and oxidants (i.e., H_2O_2).[12] EGFR transactivation is involved in metalloprotease-mediated shedding of EGF-like ligands.[9] In addition to ligand-dependent mechanisms, EGFR can be directly activated by Src phosporylation on Tyr 845. This mode of EGFR activation has been reported in association with sustained activation of EGFR in renal epithelial cells exposed to angiotensin II (Ang II).[13]

Over the past two decades, numerous studies have been conducted to elucidate the role of EGFR in renal diseases. Initial studies revealed that EGFR activation is critically involved in mediating renal regeneration and functional recovery after acute kidney injury.[14–15] Recent studies also showed that EGFR activation contributes to the pathogenesis of renal interstitial fibrosis, glomerular diseases, including diabetic nephropathy, hypertensive nephropathy, glomerulonephritis, and tubular diseases such as polycystic kidney disease.[16–20] The role of EGFR signaling in acute kidney injury and glomerular disease has been recently reviewed in other articles.[8,21] Here, we will summarize the role of EGFR signaling in renal interstitial fibrosis, and mechanisms

involved and the therapy potential of EGFR inhibition in renal fibrosis.

EXPRESSION OF EGFR AND EGFR LIGANDS IN A DISEASED KIDNEY

The location of EGFR and its ligands in the kidney has been described in the normal kidney. Constitutive EGFR expression was detected in glomeruli, tubules, and interstitium of normal human kidneys[22] and is normally expressed in several cell types in the kidney that include renal epithelial cells, podocytes, renal interstitial fibroblasts, and mesangial cells.[23] EGFR ligands, such as EGF, TGF-α, and HB-EGF are also expressed in renal tubules.[24–26] Other EGFR ligands such as amphiregulin, betacellulin, and epiregualtin are detected in the kidney at low levels, but their nephron location remains unclear.[3]

Upregulated renal expression and *de novo* expression of EGFR and its ligands has been observed in nearly all rodent-kidney injury models.[8,21] Those models include unilateral ureteral obstruction (UUO)-induced renal fibrosis,[27] Ang II-induced renal damage,[28] nephrotoxic nephritis,[23] diabetic nephropathy,[29] ischemia/reperfusion injury.[15] Cellular location of increased expression EGFR and its ligands vary with the type of kidney disease. In experimental renal fibrosis, there is an upregulated expression of EGFR by interstitial cells and renal epithelial cells.[27] In the mode of nephrotoxic serum-induced nephritis, increased EGFR was detected in podocytes and HB-EGF was in parietal epithelial cells and podocytes.[23] Tubular expression of EGFR and multiple EGFR ligands such as EGF, TGF-α, amphiregulin, and HB-EGF are also upregulated in the epithelium of a murine autosomal-recessive polycystic kidney disease model.[4] Moreover, a high level of TGF-α in renal tubules was reported in Ang II-induced renal fibrosis.[28]

Increased EGFR expression/activation under pathological conditions is either transient or persistent.[8,21] Although transient EGFR activation is seen in acute and mild injured kidneys,[8,21] its sustained expression is most observed in the kidney subjected to severe and chronic kidney damage.[13,27] In a mouse model of unilateral urethral obstruction-induced renal fibrosis, EGFR expression and phosphorylation increased gradually after urethral ligation and was persistent for at least 21 days.[27] However, EGFR activation after injury is not due solely to its increased expression, as EGFR phosphorylation is still increased even if EGFR levels are normalized.[27]

The mechanism responsible for sustained expression/activation of renal EGFR after chronic injury remains incompletely understood. We have recently demonstrated that administration of MS-275, a highly selective inhibitor of class I histone deacetylases (HDACs), reduced expression and phosphorylation of EGFR in the damaged kidney after UUO injury *in vivo* and cultured renal epithelial cells *in vitro*.[30] Gilbert *et al.*[29] also indicated that application of vorinostat, a pan-HDAC inhibitor, also resulted in reduced EGFR protein and mRNA levels, and attenuated cellular proliferation in cultured renal tubular cells. Although the molecular mechanism by which the class I HDAC inhibitor suppresses EGFR phosphorylation (activation) remains unclear, vorinostat was reported to attenuate the EGFR transcript and induce its ubiquitination and targeted it predominantly to lysosome degradation in tumor cell lines[31] In line with this observation, inhibition of a class II HDAC member, HDAC6, also accelerates segregation of EGFR from early endosomes to the late endosomal and lysosomal compartments for degradation.[32,33] These studies therefore suggest that both transcriptional and post-translational mechanisms are involved in the modulation of EGFR.

EGFR SIGNALING IN RENAL FIBROSIS

Renal fibrosis is the final common pathway in end-stage renal disease, characterized by aberrant activation and growth of the renal fibroblasts and overproduction of extracellular matrix proteins.[34] Increasing evidence indicates that activation of EGFR signaling is critically involved in the development and progression of renal fibrosis. Lautrette *et al.*[28] demonstrated that the chronic infusion of Ang II causes renal lesions such as glomerulosclerosis, tubular atrophy and/or dilation with microcyst formation, and interstitial fibrosis accompanied by severe proteinuria. These effects of Ang II were reduced in mice overexpressing a dominant-negative form of EGFR. Similar to this observation, mice overexpressing a dominant-negative EGFR construct exhibited significantly less tubulointerstitial injury in the kidney compared with wild-type littermates after subtotal renal ablation. In our study using the model of UUO-induced renal fibrosis, we found that pharmacological inhibition or genetic reduction of EGFR activity markedly reduced the expression of α-smooth muscle actin, a hallmark of activated fibroblasts, and deposition of fibronectin and collagen I, two extracelular matrix proteins, in the kidney.[16] Finally, Flamant *et al.*[35] indicated that treatment with the EGFR inhibitor gefitinib prevented the development of renal vascular and glomerular fibrosis and the decline in renal function in rats with NO deficiency–induced hypertension.

EGFR ligands have also been reported to mediate renal fibrosis and deterioration in renal function. In the model of Ang II-induced renal fibrosis, increased expression of TGF-α and its sheddase, metalloprotease-17, was observed in mice. Specific deletion of TGF-α or inhibition of TGF-α cleavage with a specific metalloprotease-17 inhibitor substantially reduced Ang II-induced renal damage,[28] suggesting that TGF-α may be a key factor between Ang II signaling and EGFR transactivation during Ang II-induced nephropathy. TGF-α expression was also increased in mice after nephron reduction in the lesion-prone FVB/N strain of mice, and EGFR inhibition reduced fibrotic lesion,[36] indicating that TGF-α acts in the genetic predisposition to chronic kidney disease (CKD) progression. In addition, sustained expression of HB-EGF in myofibroblasts during remodeling of the peri-infarct region of the remnant kidney model has also been reported,[37] suggesting a potential role for this EGFR ligand in the progression of CKD.

Signaling pathways downstream of EGFR have been studied in animal models of renal fibrosis. As stated above,

EGFR activation signals to several signaling pathways including STAT3, ERK1/2, and AKT, although EGFR is not the only receptor that mediates activation of these pathways. Our previous studies revealed that development of renal fibrosis after UUO injury is accompanied with activation of STAT3, and that blockade of STAT3 with S3I-201 reduced renal fibrosis.[38] Other studies also showed that pharmacological inhibition of ERK1/2 decreased levels of fibroblast–myofibroblast markers in the interstitium, and that inhibition of phosphatidylinositol-3 kinase reduced the number of proliferating cells and the amount of interstitial extracellular matrix deposition.[39] The mammalian target of rapamycin (mTOR) is a major downstream component of phosphatidylinositol-3 kinase. The antifibrotic effects of mTOR inhibition have been reported in several animal models of CKD, including diabetic nephropathy, chronic glomerulosclerosis, and tubulointerstitial fibrosis.[40–42] In addition, mTOR and EGFR correlation was studied in non-renal fibrotic diseases. For example, blocking mTOR with rapamycin could also attenuate TGF-α-induced pulmonary fibrosis in mice, which was similar to EGFR inhibition in this model, suggesting that mTOR is a major effector of EGFR-induced pulmonary fibrosis.[43,44] Hence, EGFR signaling may mediate renal fibrogensis and other organ fibrosis through activation of multiple signaling pathways, in particular, the phosphatidylinositol-3 kinase-mTOR signaling pathway.

MECHANISMS OF EGFR IN MEDIATING RENAL FIBROSIS

EGFR contributes to activation of TGF-β signaling

Development of renal fibrosis after injury is involved in activation of multiple signaling pathways. However, activation of TGF-β signaling is the most important mechanisms.[45] In this signaling pathway, TGF-β1 binding to TGF-β receptor II, results in the recruitment, phosphorylation, and concomitant activation of TGF-β receptor I. Activated TGF-β receptor I induces phosphorylation of Smad3, which forms a complex with Smad4 that is then translocated to the nucleus where it drives the expression of TGF-β target genes such as collagens.[45] Studies from our and other groups have demonstrated that activation of EGFR signaling is critical for increased production of TGF-β1 in murine models of renal fibrosis induced by UUO injury[16] or chronic Ang II infusion.[13] *In vitro* studies indicated that ERK1/2 act downstream of EGFR to mediate Ang II-induced sustained expression of TGF-β1.[13]

As Smad3 activation is central to TGF-β-induced fibroblast cell activation and matrix protein deposition, we also examined the effect of EGFR inhibition on Smad3 phosphorylation and found that either pharmacological or genetic blockade of EGFR reduces phosphorylation of Smad3 in UUO-induced renal fibrosis.[16] Further, exposure of cultured renal interstitial fibroblasts to gefitinib also reduced Smad3 phosphorylation and the expression of fibronectin and collagen I in response to TGF-β1 stimulation.[16] In line with this observation, Chen *et al.*[46] observed that gefitinib attenuates TGF-β1-induced cell mitogenesis via the EGFR-ERK1/2/p38 kinase pathway in cultured renal interstitial fibroblasts. These observations suggest that EGFR is coupled to TGF-β signaling to regulate its activation and mediate its biological consequences.

EGFR contributes to epithelial cells arrested in the G2/M phase after chronic injury

Epithelial cells arrested at the G2/M phase are a profibrotic phenotype that triggers production and release of TGF-β1 and connective tissue growth factor,[47] suggesting that EGFR-mediated epithelial cell G2/M phase arrest might be an important mechanism for transducing epithelial injury to renal interstitial fibroblast activation in the setting of chronic kidney injury. Although the complex cascade from epithelial cell injury to fibroblast activation and extracellular matrix protein deposition is the current subject of research, we observed that inhibition of EGFR by gefitinib or genetic reduction of EGFR decreased the number of renal tubular cells arrested at the G2/M phase, suggesting that EGFR has an essential role in this process.[16] Currently, how EGFR activation leads to G2/M arrest is not clear. Our recent research indicated that overexpression/activation of EGFR increased the expression of p21, a molecule associated with regulation of cell cycle arrested in the G2/M phase (Zhuang S, unpublished observation).

EGFR activation contributes to production of proinflammatory cytokines and chemokines after chronic injury

Kidney fibrosis is always preceded by and closely associated with chronic interstitial inflammation.[48] Multiple cytokines/chemokines including tumor necrotic factor-α and monocyte chemoattractant protein-1 are upregulated and involved in this process.[49,50] To understand the role of EGFR in their expression in the fibrotic kidney, we examined inhibition of EGFR on expression of both tumor necrotic factor-α and monocyte chemoattractant protein-1 in the injured kidney by UUO.[16] Our results showed that either pharmacological or genetic inhibition of EGFR reduced their expression,[16] indicating that EGFR activity is necessary for regulation of tumor necrotic factor-α and monocyte chemoattractant protein-1 expression. How EGFR contributes to upregulation of proinflammatory cytokines is not clear. Given that tubular epithelial cells are considered to be a prominent source of cytokines/chemokines in the kidney,[47] and EGFR-mediated epithelial cell arrested at the G2/M phase is associated with overproduction of TGF-β1 and connective tissue growth factor after chronic injury, it is assumed that EGFR signaling may also promote production of cyokines/chemokines through a mechanism involved in epithelial cell cycle arrested at the G2/M phase. This hypothesis deserves further investigation.

Taken together, sustained activation EGFR signaling regulates renal fibrogenesis through at least two mechanisms: (1) mediating activation of TGF-β signaling components (i.e., Smad3) and (2) facilitating production of profibrotic

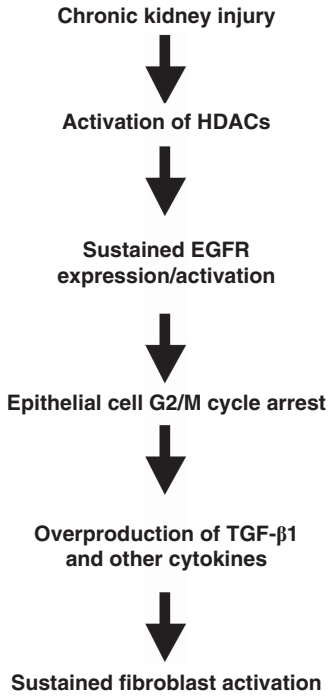

Figure 1 | Mechanisms of epidermal growth factor receptor (EGFR)-mediated expression of profibrotic factors and activation of renal interstitial fibroblasts. Following chronic kidney injury, histone deacetylases (HDACs) are activated, which induces sustained expression and activation of EGFR, and subsequently triggers epithelial cells arrested in the G2/M phase of the cell cycle. The arrested cells acquire the ability to produce high amounts of transforming growth factor (TGF)-β1 and other profibrotic cytokines, leading to activation of renal interstitial fibroblasts.

Table 1 | Approved epidermal growth factor receptors targeted therapeutics

Drug	Target	Type	Application
Gefinitib (Iressa, ZD1839)	EGFR	TKI	NSCLC
Erlotinib (Tarceva)	EGFR	TKI	NSCLC and pancreatic cancer
Cetuximab (Erbitux)	EGFR	Chimeric mAb	CRC, and head and neck cancer
Panitumumab (Vecitibix, ABX-EGF)	EGFR	Fully human mAb	CRC
Trastuzumab (Herceptin)	ErbB2	Humanized mAb	Breast cancer

Abbreviations: CRC, colorectal cancer; EGFR, epidermal growth factor receptors; mAb, monoclonal antibody; NSCLC, non-small-cell lung carcinoma; TKI, tyrosine kinase inhibitor.

factors (i.e., TGF-β1). The later mechanism is summarized in Figure 1.

POTENTIAL IMPLICATIONS OF EGFR AS A THERAPEUTIC TARGET IN KIDNEY DISEASES

Excessive signaling of EGFR is a hallmark of a wide variety of solid tumors. Several studies have also demonstrated increased expression of EGFR and its ligands in the fibrotic kidney, and the beneficial effect of EGFR inhibition in attenuating development of renal fibrosis in a variety of animal models.[16,28,30,50] Thus, EGFR may be an attractive candidate for targeted therapeutic treatment of renal fibrosis. To date, several anti-EGFR therapies have been developed (Table 1), and the clinical application of EGFR inhibitors for patients with high levels of EGFR expression in non-small-cell lung cancers suggests that blockade of EGFR with these inhibitors may also be effective in treating CKD in humans. Because EGFR activation and EGFR-induced cellular events are mediated by downstream signaling pathways, therapies inhibiting various aspects of the EGFR signaling cascade may be also beneficial in alleviation of CKDs. In this regard, pharmacological inhibition of ADAM metallopeptidase domain 17, a protease that mediates cleavage of the mature form of EGFR ligands from membrane precursors, also attenuates renal lesions induced by Ang II infusion.

Owing to expression of EGFR in epithelia under a physiological state, side effects are noted in anti-EGFR therapy such as skin toxicity. Other side effects include nausea, vomiting, loss of appetite, diarrhea, dryness, itching, acne, and weakness.

SUMMARY

Beyond its fundamental role for kidney development and physiology, the EGFR signaling is implicated in many pathologic conditions including renal fibrogenesis.[13,16,28,51] Excessive EGFR signaling induces renal fibrosis through increasing TGF-β1 expression,[16,28] regulating activation of TGF-β signaling,[16] promoting epithelial cell G2/M arrest,[27] and enhancing production of inflammatory cytokines/chemokines.[16] Given that EGFR is activated by ligand-dependent and -independent mechanisms and mediates fibrotic responses, EGFR could serve as convergent platform for diverse insults to induce/promote renal fibrogenesis. On this basis, it is envisioned that blockade of EGFR signaling pathways would have therapeutic potential for halting or slowing down progression of renal fibrosis in kidney diseases.

DISCLOSURE

The authors declared no competing interests.

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